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Sendai
ISSF97
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PROCEEDINGS

The 4ᵗʰ International Symposium on Supercritical Fluids

Volume C

Oral Presentations

Sendai, Japan

11 - 14 May, 1997

Extraction, Fractionation, and Reaction of Agricultural, Food and Natural Products Utilizing Supercritical Carbon Dioxide and Cosolvent Mixtures

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The application of supercritical fluid extraction (SFE) as a singular process technique for the isolation and refinement of complex natural and agriculturally-derived products has inherent limitations. The molecular complexity of these mixtures requires additional post extraction refinement and/or conversion that can be accomplished with the aid of supercritical fluids via such processes as supercritical fluid fractionation (SFF) and supercritical fluid reactions (SFR). In this presentation, we report on our studies utilizing SFE, SFF, and SFR in tandem to produce final products for both industrial and food uses. To optimize the above processes, novel micro SFE and catalyst evaluation techniques have been developed using analytical scale SFE equipment. The efficacy of both chemical and biochemical catalysts in the presence of supercritical fluid media have been measured.

The utility of lipase catalysts has been demonstrated for producing fatty acids, esters, mixed glycerides, and interesterified oils. Utilizing another environmentally-compatible fluid, subcritical water, we have successfully hydrolyzed vegetable oils to their corresponding fatty acid mixtures. Optimization SFE studies for cedarwood oil removal and vernonia seed extraction are also reported. Glyceride mixtures derived from two different reaction schemes in the presence of supercritical carbon dioxide (SC-CO_2) can be enriched in their monoglyceride component by the application of a thermal gradient fractionation column operating under supercritical conditions. This SFF process as well as supercritical fluid chromatography (SFC) offer numerous opportunities for creating combined extraction and enrichment sequences, all conducted with the aid of supercritical fluid media.

Introduction

The utilization of supercritical fluids for processing agri- and natural products involves more than simple SFE, and today includes fractionation and reaction schemes that are frequently integrated into a basic extraction protocol to achieve a desired end result. This trend is due to the increasing complexity of many naturally-derived products and the desire to implement "green" chemistry and manufacturing processes [1,2] in isolating and synthesizing a final product. In addition, the high capital equipment costs associated with implementing the use of supercritical fluids in industry suggest that the fluid source be used in as many unit processes as possible, including, but not restricted to; extraction [3], fractionation [4], reaction [5], product protection and/or modification [6].

As shown in Fig. 1, there are numerous possibilities for combining supercritical fluid-based processes to achieve the desired end. Figure 1 indicates that as opposed to using SFE to yield a desired end product from "nature", that the resultant extract can be further treated by employing SFF or SFR to yield fractions enriched in a particular component or the final end products. The sequences noted in Fig. 1 are partially based on research conducted in our laboratory. For example, seed oils have been extracted with SC-CO_2 to yield predominately a triglyceride-rich extract that is further reacted to yield products such as

esters [7], mixed glyceride species [8], or modified oils [9]. Another alternative is to combine SFE with SFF to yield targeted end products, which we have demonstrated by utilizing a SC-CO_2 density gradient in a packed column to fractionate glyceride mixtures [10], or by employing preparative supercritical fluid chromatography (SFC) for the concentration of tocopherol-rich mixtures from seed oil matrices [11].
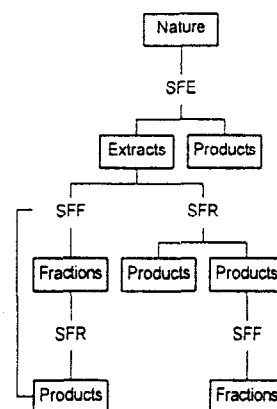

Figure 1. Process Sequences Utilizing Supercritical Fluid Media for Isolating or Synthesizing Desired End Products.

In this presentation we shall report on our studies that are designed to integrate the use of supercritical as well as subcritical media for processing compounds derived from agrichemical and natural products. We shall focus on three subject areas: (1) the development of supercritical fluid-based sequences employing SFE in combination with SFF and SFR, using both flow and batch processing, (2) new and novel techniques and apparati for evaluating and optimizing the above processing steps, and finally (3) examples which illustrate the multiple uses of catalysts in the presence of SC-CO_2 to produce an array of desired end products [12].

Materials and Methods

Many of the processes cited here have already been described in the literature or will be published shortly in available journals, hence in the interest of space, we have elected to focus on the new techniques and apparati that are used to optimize the supercritical fluid processes or evaluate catalytic activity in the presence of the supercritical fluid. The glycerolysis reactions conducted in the presence of SC-CO_2 and water were run using a standard batch, stirred autoclave [13], while those initiated in the presence of the lipase, Novozym SP 435 (Novo Nordisk, Danbury, CT), were facilitated using multiple syringe pumps (Isco, Inc., Lincoln, NE) and an Isco SFX 2-10 extraction module which contained the small extraction cells used in the experiments. Scale up of the latter reaction sequence employed home built extraction/reaction equipment previously described in the literature [14]. The fractionation of the mixed glyceride mixtures noted above were conducted on a packed column [10] using multiple temperature zones running from 65 to 95°C. Further details can be found in these proceedings as described by the poster presentation of Sahle-Demessie [15].

A new method for testing catalyst effectiveness in the presence of SC-CO_2 involves recirculating the dissolved reactants in the fluid over a small bed of catalyst. The apparatus used in these studies consisted of a modified Milton Roy SPA system (Milton Roy, Riviera Beach, FL) as shown in Fig. 2. Here the catalyst to be tested is inserted into the catalyst vessel downstream of the reactant vessel. The system utilizes a high pressure piston pump to pressurize and fill the system with CO_2, including a recirculation loop that passes the CO_2 at a constant flow rate through both the reactant and catalyst vessels. Samples can be taken intermittently through a side stream sample valve/loop arrangement (Fig. 2) by washing the decompressed extract out of the loop with an auxiliary sample pump.

Five catalysts types have been studied, including the above-mentioned lipase, an acidic cation exchange resin, p-toluene sulfonic acid, an acidic alumina, and a high temperature esterification catalyst (TiS) from Engelhard Industries (Newark, NJ). Typically, catalyst activities were
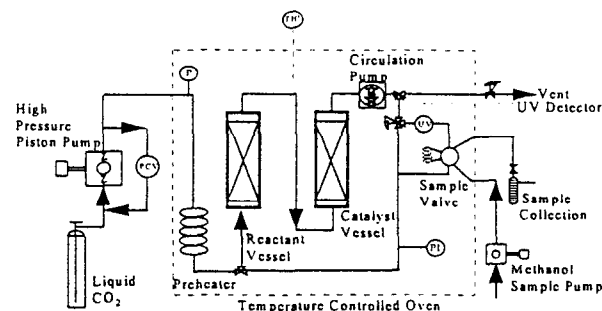

Figure 2. Recirculation Reactor Utilized for Evaluating Catalysts Under Supercritical Fluid Conditions.

evaluated by running model esterification reactions at 20.7 or 45.5 MPa and 70°C. Methanolysis was performed on palmitic, stearic and oleic acids under the above conditions, and the effect of fatty acid/catalyst ratio evaluated.

Hydrolysis of vegetable oils to produce fatty acid mixtures is another environmentally-compatible process that can utilize sub- or supercritical water media. Initial experiments to optimize the reaction conditions were conducted in a small batch tubular autoclave (11 or 35 ml volume) at temperatures ranging from 250 to 375°C, with the water density varying from 0.5-0.7 g/ml. Further details on this particular experimental apparatus are described in the literature [16]. Recently, these studies have been extended to utilize a flow reactor as shown in Fig. 3. In this apparatus, both water and vegetable oil are pumped into the flow reactor with the aid of syringe pumps (Isco, Inc., Lincoln, NE) before entering the reaction chamber (RC). The products and water are then cooled (CB) and decompressed into a collection vial (CV).
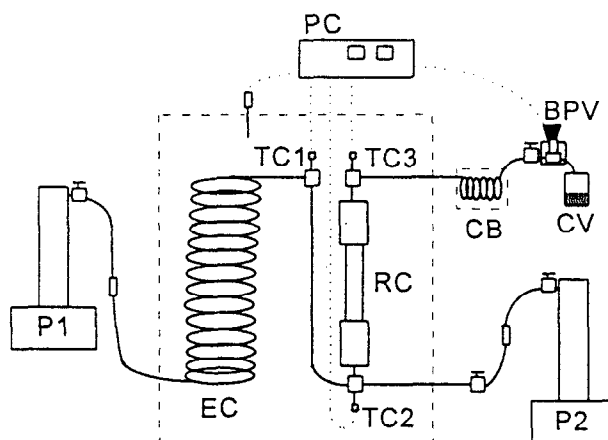

Figure 3. Schematic of Water Reaction System. P1 (Isco 260 D syringe pump for water), P2 (Isco 100 DX pump for oil), EC (equilibrating coil), TC1-3 (Type K thermocouples), PC (process controller), RC (reaction chamber), CB (cooling bath), BPV (backpressure valve), CV (collection vial).

The extractions of cedarwood oil were performed sequentially and automatically on an Isco Model SFX 3560 extractor. This permitted individual samples of the wood matrix to be extracted using different combinations of pressures, temperatures, and extraction times as designated via the microprocessor controller on the extractor. For example, a matrix of three different temperatures and four different pressures was tested at a constant flow rate (2.0 ml/min LCO_2) with respect to oil yield and chemical composition. In addition, the rapid sample processing capabilities of the Model SFX 3560 permitted the total extraction curve (mass of oil vs. time) to be determined where each individual data point was generated on a separate sample of cedarwood. The advantage of this method is that it avoids the introduction of any bias into the extraction rate curve that might occur by using just one sample of wood. It is also possible, by using the microextractor, to limit the amount of sample required for optimizing the extraction conditions, an important feature when studying limited amounts of natural products.

Extractions of *Vernonia galamensis* seed were conducted on a commercial Applied Separations Spe-ed system (Applied Separations, Inc., Allentown, PA) using SC-CO_2. Extractions were conducted over the pressure range 13.8 - 69 MPa and temperatures ranging from 40 - 100°C. The bench scale extractor allowed the use of small quantities of vernonia seed (approx. 5 g/run) which was in limited supply. Exhaustive extractions were not conducted since the aim of these experiments was to optimize parameters that would maximize oil yield, reduce the total fatty acid content, and lead to enriched fractions containing vernolic acid. With respect to minimizing the total fatty acid content in the collected extracts, conditions were sought that would deactivate the lipase in the seed that leads to the production of free fatty acids. In order not to activate the lipase, seed samples were comminuted in the presence of dry ice, prior to SFE.

Results and Discussions

Production of Chemicals Via Lipolysis of Oilseeds. As illustrated in Fig. 4, it is possible using a common lipase as a catalyst, to initiate the production of several different types of chemical compounds from oilseeds. Using Novozym SP 435, we have been able to perform simple esterifications, transesterifications, oilseed glycerolysis, and interesterifications on triglyceride-bearing substrates. The key to these natural product transformations is control of the water content during the reaction in the presence of SC-CO_2, and tuning the reaction conditions so that lipase activity is not compromised at a particular temperature or pressure.

Toward this end we have been able to perform methanolysis of seed oils successfully at 24.1 MPa and 50°C and achieved methyl ester conversions in excess of 98% from corn and soybean oil [7]. These conversions are
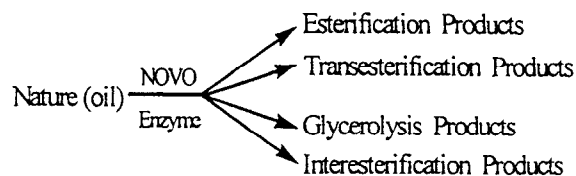

Figure 4. Transformations of Natural Oils via Enzymatic Catalysis in SC-CO_2.

sensitive to the methanol injection rate and can be maximized only in the presence of water below 0.1 % v/v. Glycerol does not inhibit the reaction and can be collected in the receiver vessel after decompression of reaction mixture from the flowing SC-CO_2 stream. Interestingly, this reaction can be used for analytical purposes to determine the fat content of foods and oilseeds [17].

Glycerolysis can also be conducted in the presence of flowing SC-CO_2 and a lipase catalyst, although evidence points to the transformation being conducted in a biphasic system. Jackson and King [8] have recently reported the reaction of glycerol, 1,2-propanediol, ethylene glycol and methanol with soybean oil in a flow reactor that uses multiple syringe pumps to add the alcoholic reagent and oil into the SC-CO_2. Optimal temperature and pressure for glycerolysis were found to be 27.6 MPa and 70°C, respectively. By adjusting the water content of the glycerol and flow rate of the soybean oil, one can regulate the level of the monoglycerides that are produced. For example, addition of 0.7 % w/w water to the glycerol feed resulted in an 84% conversion of oil to monoglycerides at the above reaction conditions. Conducting such a synthesis in the presence of SC-CO_2 can obviously impart considerable flexibility in tailoring the reaction to yield a specific target end composition.

Lipase-initiated interesterifications have also proven possible in the presence of SC-CO_2. This can be achieved by solubilizing the oil(s) to be interesterified in SC-CO_2 and passing the mixture over the Novozym SP 435 at 65°C and pressures from 17.2 - 41.4 MPa [9]. The resultant oils have dropping points and solid fat indices that depend on the amount of catalyst, and to a lesser extent on CO_2 flow rate and pressure. More importantly, the above method can yield randomized fat compositions that can be utilized as potential candidates for incorporation into margarine formulations.

SFF of Glyceride Mixtures. Glycerolysis can also be conducted in the presence of SC-CO_2 in a traditional batch reactor where the CO_2 acts as a catalytic agent replacing the use of metal catalysts [13]. We have found that this synthesis can be conducted to advantage in SC-CO_2 and that optimal results are achieved at a pressure of 20.7 MPa and 250°C, a glycerol to water ratio of 25, with 4% addition of water. Depending on the chosen oilseed substrate, monoglycerides can be produced at 40 - 50%

wt. % levels, with the competing hydrolysis reaction usually yielding between 10 - 15 wt. % fatty acids. The resultant glyceride mixtures tend to be lighter in color and less odoriferous than those derived from traditional manufacturing methods.

Such glyceride mixtures, whether derived from the above method or lipase catalysis, can be further enriched in monoglyceride content via traditional fractionation procedures, or alternatively by applying SFF. Using the previously described thermal gradient fractionation column [10,15], monoglyceride enrichments exceeding 90 wt. % can be achieved. This enrichment is best carried out at lower densities of SC-CO$_2$ as shown in Fig. 5. Selectivity for monoglyceride drops as the CO$_2$ density is increased, thus there is a tradeoff between selectivity and throughput that can only be overcome by increasing the capacity of the fractionation column. Nevertheless, the concept of using SFF coupled with SFE or SFR is attractive, and in the case of monoglyceride enrichment, yields products that are equal to those derived via vacuum distillation.
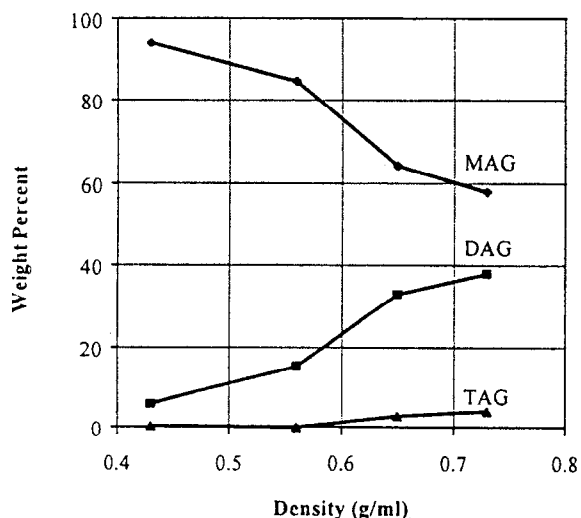

Figure 5. Dependence of Glyceride Fractionation on CO$_2$ Density.

Evaluation of Catalysts for SFR. Microextraction modules normally used for analytical SFE have proven very versatile with respect to evaluating catalytic activity in the presence of SC-CO$_2$ and its mixtures with cosolvents (reactants) or water. Using the Isco SFX 2-10 system, we have shown that the Novozym SP 435 catalyst can be used at temperatures up to 70°C and pressures of 35 MPa; the optimal conditions varying somewhat with respect to what type of reaction being conducted and the specific system that is being extracted.

Recently, we have employed a recirculation reactor to test the effectiveness of several diverse types of catalysts in performing model esterification reactions. Table 1 summarizes this type of evaluation for the reaction between oleic acid and methanol at 34.5 MPa and 70°C for 5 types

of catalysts using a mass ratio of fatty acid/catalyst equal to 5 and a run time of 2 hrs. As Table 1 indicates, the very acidic catalyst, p-toluene sulfonic acid, outperforms all of the other catalysts although a 70% conversion can be achieved using the Novozym SP 435 under equivalent reaction conditions. The above esterification reactions were all first order with respect to time, the product yields being determined by the solubility of the reactants in SC-CO$_2$ and the efficacy of the catalysts as shown in Table 1.

Table 1. Esterification of Oleic Acid and Methyl Alcohol in SC-CO$_2$ by Various Catalysts.

Catalyst	Conversion
Titanium Silicate	6.4
Cation Exchange Resin	13.4
Acidic Alumina	13.0
Novozyme 435	69.8
p-TSA	99.7

$M_{FA}/M_{Cat} = 5$
$T = 70°C$ $P = 3000$ psi (34.5 MPa)
Time = 2 hrs

Hydrolysis of Oilseeds in Water. Initial batch experiments have indicated that hydrolysis could be successfully conducted under subcritical water conditions without deleterious degradation of the fatty acid product. Using temperatures of 260-280°C, we were successful in obtaining fatty acid conversions greater than 97%. These conversions were achieved at a water density of 0.7 g/ml over a 15-20 min. time interval for 4 individual oilseeds. The use of higher temperatures gave some degraded product and led to the isomerization of linolenic acid. After these initial optimization runs, a flow reactor was used to convert soybean oil to its corresponding fatty acids. As shown in Table 2, the resultant conversions are dependent on the respective residence time (i.e., pumping rates) of the oil/water phase. The described dynamic extraction/reaction system is very versatile and can be used as a model system for further process development. Such a future scheme could entail the use of SC-CO$_2$ to separate the fatty acids from the emulsified water-lipid phase.

Optimizing the SFE of Cedarwood Oil. Cedarwood oil is a natural product which finds utility as an ingredient in cosmetics, as an insect repellent, and odorant. The cost of cedarwood oil is approximately $13/kg making its economic value compatible with utilizing SFE for its isolation. To optimize its extraction, experiments were

Table 2. Conditions Needed to Obtain >97% Conversion of Vegetable oils to Fatty Acids in Subcritical Water.

Vegetable Oil	Time (min)	Temp (°C)	Press (MPa)
Soybean Oil (RBD)[a]	20	270	Batch (15.2 calc)
Hydrogenated Soybean Oil	15	280	"
Linseed Oil	20	280	"
"	69	260	"
Coconut Oil	15	270	"
Soybean Oil (RBD)	12.5[b]	260	12.6
"	9.8[c]	260	13.1
"	14.8[d]	255	12.6

a) Refined, bleached, deodorized soybean oil.
b) 2.0mL/min water, 0.8mL/min oil.
c) 3.0mL/min water, 0.8mL/min oil.
d) 2.0mL/min water, 0.4mL/min oil.

run on a commercial automated microextraction apparatus from 27.6 MPa - 69 MPa using temperatures of 40, 70 and 100°C. As shown in Table 3, the maximum yield of oil plus water is achieved at a temperature of 100°C, however the effect of pressure at all of the temperatures tested had a negligible effect on extract yield. Similarly, the composition of the chemical components in the extracted cedarwood oil remained relatively constant with respect to the extraction pressures and temperatures tested. It should be noted that the extraction data presented in Table 3 were determined in less than 8 hrs., using the automated analytical extractor.

Table 3. Supercritical CO_2 Extraction of Cedarwood Chips: Efficiency and Component Ratios as a Function of Temperature and Pressure.

Temp (°C)	Pressure (MPa)	Weight % Collected	Cedarwood Oil Composition (%)		
			Cedrene	Thujopsene	Cedrol
40°C	27.6	2.9	0.8	1.0	85.3
40°C	41.4	3.1	0.9	1.1	84.4
40°C	55.2	2.4	1.1	1.3	83.3
40°C	69.0	2.9	1.0	1.2	84.6
70°C	27.6	4.7	1.3	1.3	84.8
70°C	41.4	5.4	1.3	1.4	84.1
70°C	55.2	6.3	1.3	1.3	83.8
70°C	69.0	5.9	1.4	1.3	83.9
100°C	27.6	8.0	1.8	1.4	84.3
100°C	41.4	7.9	1.8	1.4	83.1
100°C	55.2	8.2	1.8	1.4	84.6
100°C	69.0	8.2	2.0	1.5	83.0

Likewise, extraction rate curves can also be determined quickly using the microextractor. Figure 6 shows the extraction of cedarwood at 27.6 MPa at 100°C. Each point in this extraction curve was determined on an individual sample of cedarwood (approx, 2g), thereby eliminating any bias due to sample heterogeneity. The data shows that most of the oil content of the cedarwood is removed in a 20 min. time period. This technique has many advantages, namely the use of small quantities of a natural product as well as the ability to rapidly determine the extraction characteristics of different sources of cedarwood or any other natural matrix.
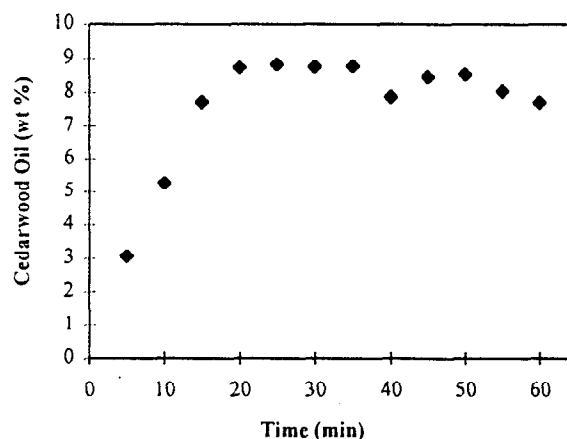

Figure 6. Rate of Extraction of Oil from Cedarwood.

SFE of Vernonia Seeds. Extractions of crushed vernonia were conducted using a matrix of pressures (13.8, 34.5, 69 MPa) and temperatures (40, 60, 80, 100°C). The vernolic acid and total free fatty acid content of the extract, oil yield, and lipase activity of the deoiled seeds were determined by our collaborator, Professor Ali Mohamed of Virginia State University (Petersburg, VA). Oil extraction was found to be maximized at higher temperatures and pressures, which has been found previously for the SFE of triglyceride-containing oilseeds [18]. The vernolic acid content in the extracts ranged from 43 to 63% of the theoretical yield. Vernolic acid is an epoxy-containing fatty acid of industrial importance. For extractions conducted at 40°C, it was found that lipase activity still remained in the vernonia seed as judged from the fatty acid content in the residual oil of the partially extracted oilseed. From these screening experiments, a significant reduction in the total free fatty content was found in going from low pressures and temperatures to high pressures and temperatures, e.g., 13.8 MPa at 40°C to 69 MPa at 100°C. This yielded extracts having 69 versus 8 mg - free fatty acid/g - oil , respectively.

Conclusions

In this presentation it has been demonstrated that processes such as extraction, fractionation and reaction can be conducted in supercritical fluid media successfully, leading to the potential for combining such processes in sequence to yield an entire manufacturing process that is environmentally -compatible. Using carbon dioxide and water, we have demonstrated that a variety of lipid- based chemicals can be produced or transformed into industrial products. Factors that must be considered in designing such processes and their optimization are the solubility of the compounds or reactants in SC-CO_2 or subcritical water, the activity of catalysts in the presence of supercritical fluids, and engineering the sequencing of the individual SFE, SFF and SFR steps. Future work will include using SFC in conjunction with SFE and SFF and possibly a CO_2-based recovery process coupled with reaction chemistry in sub- and supercritical water.

Acknowledgments

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